

Amendments to the Specification

Please replace paragraph 0102 with the paragraph below.

[00102] To compare the efficiency of transfection of siRNA in the presence of dendrimers or a standard transfection agent, 21-nucleotide 5'-Cy3-labeled CDK9 sense strand siRNA was deprotected and annealed to unmodified antisense strand and purified as described above. HeLa cells were maintained at 37C in Dulbecco's modified Eagles medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100 µg/ml streptomycin (Invitrogen). Cells were regularly passaged at sub-confluence and plated on 60mm plates 16 hr before transfection at 70% confluency. As a control, 20 µg LipofectamineTM (Invitrogen)-mediated transfections of 100 pmole CDK9 5' Cy3-SS/AS duplex siRNAs were performed in 60mm plates as described by the manufacturer for adherent cell lines. For comparison, CDK9 5' Cy3-SS/AS duplex siRNAs were also transfected by mixing with various amount of PAMAM (Sigma-Aldrich) (ranging from 10 µg to 1 mg) using the same conditions as for LipofectamineTM-transfection. Cells were incubated in 1 ml of transfection ~~the transfection~~ mixture for 6 hours and washed three times with PBS (Invitrogen) to remove the transfection mixture. Total nucleotide including DNA, RNA and the transfected siRNAs were isolated by RNA/DNA minikit (QIAGEN) and precipitated by isopropanol. After being dissolved in DEPC-treated water, nucleotide mixtures were subjected to fluorescence measurements on a PTI (Photon Technology International) fluorescence spectrophotometer. The slits were set at 4 nm for both excitation and emission lights. All experiments were carried out at room temperature. Fluorescence of CDK9 5'Cy3-SS/AS duplex siRNA was detected by exciting at 550nm and emission spectrum was recorded from 560nm to 650nm. As shown in FIGS 1A and 1B, the spectrum peak at 570 nm represents the fluorescence intensity of Cy3, which is an indicator of the uptake of CDK9 5'Cy3-SS/AS duplex siRNA as well as the siRNA transfection efficiency by using LipofectamineTM or various amount of PAMAM. The results shown in FIG. 1A indicate the successful transfection of CDK9 5'Cy3-SS/AS duplex siRNA into HeLa cells by PAMAM (dendrimer). The fluorescence of CDK9 5'Cy3-SS/AS duplex siRNA was detected by exciting at 550 nm and emission spectrum was recorded from 560 nm to 650 nm. For control, results from cells subjected to LipofectamineTM-mediated transfection are also shown in black line in FIG. 1A. FIG. 1B is a comparison of siRNA transfection efficiency mediated by PAMAM

(dendrimer) to LipofectamineTM. The bars represent the spectrum peak at 570 nm from FIG. 1A (the fluorescence intensity of Cy3, which is an indicator of the uptake of CDK9 5'Cy3-SS/AS duplex siRNA as well as the siRNA transfection efficiency) for each of the listed conditions.

After normalizing the Cy3 signal for PAMAM transfection with the Cy3 signal derived from LipofectamineTM-mediated transfection, 20-40 µg PAMAM (dendrimer) (bars 3 and 4) is nearly equal to that of 20 µg LipofectamineTM (bar 1). Using higher amounts of PAMAM may interfere with the siRNA uptake (bars 5-8). These results demonstrate that PAMAM can be used to deliver siRNAs efficiently into living cells.